Synthetic Elastase Inhibitors: Prospects for Use in the Treatment of Emphysema[1-3]

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SUMMARY Human polymorphonuclear leukocyte elastase is the enzyme primarily responsible for the destruction of lung tissue observed in pulmonary emphysema. A number of potent reversible and irreversible inhibitors have been developed for human leukocyte elastase. Several of these inhibitors have been shown to be effective at preventing emphysema in animal models of the disease. There are excellent prospects for the development of a synthetic elastase inhibitor for use in treatment of human disease. AM REV RESPIR DIS 1983; 127:S54-S58

Introduction

Human leukocyte (HL) elastase is suspected by many investigators to be the primary destructive agent in pulmonary emphysema. Other tissue proteases such as cathepsin G (1), the serine proteases from mast cells (2), and macrophage metallo-elastase may contribute to the damage. Indeed, cathepsin G appears to act synergistically with HL elastase and aids slightly in the solubilization of elastin ([3] and unpublished observations). However, these other enzymes must be given minor roles at best because their natural serum inhibitors (α_1-antichymotrypsin for cathepsin G and the mast cell chymotrypsin-like enzymes, and α_2-macroglobulin for the macrophage elastase) appear to be unaffected in individuals with emphysema.

α_1-Protease Inhibitor

The reactive site of α_1-PI has an Ala-Ile-Pro-Met*Ser-Ile-Pro-Pro sequence (4). The mechanism by which α_1-PI inhibits elastase and other serine proteases is not yet known, but it certainly involves interaction of the reactive site amino acids of α_1-PI with the active site of the protease. It is possible that the active site serine of elastase reacts covalently with the Met-Ser bond of α_1-PI to form a tetrahedral complex, an acyl enzyme, or simply interacts with no covalent bond formation, but this has not been definitely established. When complexes of proteases and α_1-PI are dissociated at high pH or by using nucleophiles (5), the Met-Ser bond indicated by an asterisk in the above sequence is cleaved.

We have synthesized a number of linear peptides with the amino acid sequence at the α_1-PI reactive site and shown them to be perfectly adequate substrates for HL elastase (6). The octapeptide Ac-Ala-Ile-Pro-Met-Ser-Ile-Pro-Pro-NH_2 is not an inhibitor of elastase but is an excellent substrate and is hydrolyzed at the Met-Ser bond with a K_M = 1.6 mM and k_{cat}/K_M = 10,000 $M^{-1}s^{-1}$. Oxidation of the Met in the octapeptide to methionine sulfoxide in Ac-Ala-Ile-Pro-Met(O)-Ser-Ile-Pro-Pro-NH_2 resulted in a twofold increase in K_M(K_M = 3.6 mM) and a 370-fold decrease in k_{cat}/K_M = 27 $M^{-1}s^{-1}$ at pH 7.5. Similar results have been obtained with other Met-containing peptides (7).

Therapy

Several approaches to treatment of emphysema can be envisioned. These include (*1*) use of α_1-PI, (*2*) use of other natural high molecular weight protease inhibitors from either plant or animal sources, (*3*) use of antioxidants to protect the α_1-PI of smokers (the role of oxidative processes in inactivating α_1-PI is discussed elsewhere in this symposium), and (*4*) use of low molecular weight elastase inhibitors. The use of α_1-PI in replacement therapy for PiZ patients has been reported (8) and is discussed in other reviews in this symposium. A number of other protease inhibitors that have been shown to inhibit HL elastase (9) also are candidates for this type of therapy. The disadvantage of these methods is the lack of oral activity of the protein protease inhibitors that are unlikely to survive and be absorbed from the digestive system. In addition, it probably will be difficult to obtain sufficient supplies of α_1-PI to treat the required number of patients with emphysema. The use of antioxidants to protect smokers appears to be a viable option, but has not been widely studied.

Low molecular weight elastase inhibitors have a number of advantages: (*1*) they could be obtained in sufficient quantities either by chemical synthesis or fermentation, (*2*) synthetic inhibitors could be rationally designed, (*3*) they would be non-antigenic, (*4*) they could be used orally or in aerosols, and (*5*) they would be readily absorbed and transported to the site of action. On the other hand, each new structure would require extensive toxicity testing in animals before approval could be obtained from the FDA.

Synthetic elastase inhibitors can be designed to bind either reversibly or irreversibly to elastase. Irreversible inhibitors have the advantage that elastase usually cannot become reactivated once the inhibition reaction takes place. Reversible inhibitors in contrast always must be present in sufficient concentration, and as the inhibitor is degraded or excreted, the elastase would regain its activity. A disadvantage of irreversible inhibitors is their possible increased toxicity due to reaction with other proteins in the physiologic medium. Irreversible inhibitors often contain reactive functional groups that can react with many side-chain functional groups of proteins.

It is conceivable that an elastase inhibitor could be targeted only for extracellular enzyme. This could be done by making alterations in the inhibitor structure that prevent or slow membrane transport. Such an inhibitor would be ideal for treating emphysema because HL elastase is only destructive outside of leukocytes. A targeted elastase inhibitor would prevent the deleterious effects of elastase although not altering its normal physiologic function.

Human Leukocyte Elastase

The elastase found in the granule fraction of human polymorphonuclear leukocytes is a typical serine protease belonging to the same family as more widely studied enzymes such as trypsin, chymotrypsin, and porcine pancreatic elastase. At present only small parts of the HL elastase sequence have been determined (10) and no X-ray crystallographic studies have been undertaken. Thus classification of HL elastase as a serine protease has been extremely valuable in terms of rational inhibitor design. Within a family of proteases, the catalytic residues in the active site of each member of the family are likely to be identical or very similar in structure and geometry. X-ray crystallographic studies on several serine proteases has shown this to be true even where the individual proteases such as chymotrypsin and subtilisin have evolved from different precursors. Thus it is possible to derive a fairly complete working model of the catalytic site of HL elastase from studies that have been carried out with better known members of the serine protease family such

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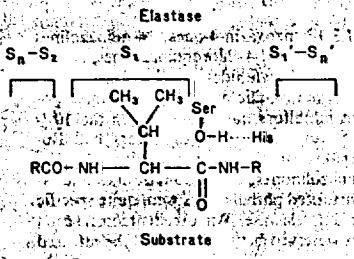


Fig. 1. A schematic drawing of the binding of a valine peptide substrate to the active site of HL elastase. The serine and histidine are 2 of the catalytic residues and the S_1 pocket is the primary substrate recognition site of the enzyme. S_n–S_2 and S_1'–S_n' are subsites that recognize other amino acid residues in an extended peptide substrate.

as bovine trypsin and chymotrypsin. This has allowed us and other investigators to direct inhibitors against specific functional groups in elastase's active site before sufficient enzyme or time was available to prove that those functional groups are present.

The substrate specificity of elastase is a poor predictor of the nature of its active site. Elastolytic enzymes are known that belong to at least 2 of the 4 protease families. These include serine proteases such as HL and porcine pancreatic elastase, and metalloproteases such as macrophage elastase and the elastase from *Pseudomonas aeruginosa*. In addition, enzymes such as thermolysin that are not usually considered elastases have high elastolytic activity. Indeed, classification on an elastase is not even informative in regard to the bonds cleaved in elastin because HL elastase and *P. aeruginosa* elastase have quite different substrate specificities.

A schematic representation of the active site of HL elastase is shown in figure 1. Based on crystallographic studies with other serine proteases (11), the catalytic residues are composed of a triad of amino acid residues, serine, histidine, and aspartic acid (not shown), that actually perform the peptide bond hydrolysis. In addition to the catalytic residues, the active site is composed of an extended substrate binding site that consists of a primary substrate binding site S_1 and various subsites on either side of the scissile bond. These subsites contribute to the binding of extended peptide substrates or inhibitors. The extended substrate binding site of HL has been shown to encompass at least 7 or 8 amino acid residues and has an equal number of subsites (6). The primary substrate binding site S_1 of HL elastase prefers to bind with substrates or inhibitors containing valine residues (12, 13). Porcine pancreatic elastase has a much smaller binding site and prefers alanine residues rather than valine.

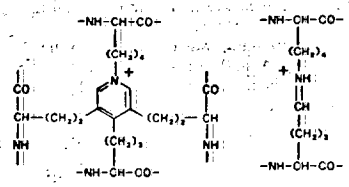
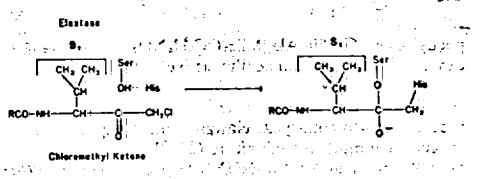


Fig. 2. Structure of 2 common elastin cross-linking residues, desmosine and dehydrolysinonorleucine. Isodesmosine is an isomer of desmosine where the substituents are attached to the 1, 2, 3, and 5 positions of the pyridinium ring.

Fig. 3. A schematic drawing of the reaction of a valine chloromethyl ketone with the active site of HL elastase. The enzyme is alkylated at the imidazole ring of the active site histidine residue. In addition, the serine OH adds to the carbonyl group of the inhibitor.



HL elastase effectively cleaves elastin, the flexible, highly cross-linked protein that is found in high quantities in mammalian lung and arteries. The chemical structure of elastin is not yet known, but fragmentary data available indicate that elastin has long sequences of small aliphatic amino acid residues such as Gly, Ala, Val and Pro. It also contains a number of cross-linking amino acid residues such as desmosine and isodesmosine (figure 2). These cross-linking residues are formed by the action of lysyl oxidase upon Lys residues in tropoelastin, a precursor of elastin. Studies with synthetic substrates containing model desmosine residues (14) support the hypothesis that HL elastase is a cross-link selective enzyme. In particular, HL elastase prefers to cleave sequences such as cross-linking amino acid residue $(AA)_n$-P_1*P_1' (asterisk indicates bond cleaved) where P_1 is Val or Ala and n is 1 or 2 but not zero. HL elastase also binds very tightly to elastin and the evidence indicates that this involves the cross-linked regions of elastin. In addition, it has been shown recently that HL elastase preabsorbed onto elastin is incompletely inhibited by α_1-protease inhibitor (15).

Human Leukocyte Elastase Inhibitors

Peptide chloromethyl ketones. The first class of selective inhibitors to be developed for HL elastase were peptide chloromethyl ketones (13, 16). These are active site-directed irreversible inhibitors for serine proteases. The inhibitors bind to the extended substrate binding site of the enzyme, and the reactive chloromethyl ketone functional group is then placed in the proper position to alkylate the active site histidine residue. In addition, the serine OH reacts with the inhibitor carbonyl group to form a hemiketal. A schematic drawing of the inhibition of HL elastase by a chloromethyl ketone is shown in figure 3.

The most effective chloromethyl ketone HL elastase inhibitor found thus far is MeO-Suc-Ala-Ala-Pro-ValCH_2Cl($k_{obs}/[I]$ = 1560 $M^{-1}s^{-1}$, MeO-Suc = $CH_3OCOCH_2CH_2CO-$) (13). This will not inhibit the other major leukocyte protease cathepsin G, but is an alkylating agent and will react slowly with nucleophiles such as glutathione ($k_{obs}/[I]$ = 0.88 $M^{-1}s^{-1}$). This rate is quite slow and the inhibitor would discriminate in favor of HL elastase over glutathione by a factor of 1700 if the concentrations were equivalent.

The major problem with the use of peptide chloromethyl ketones *in vivo* is the potential for reaction at other sites. One possible approach to obtaining specificity involves the attachment of the inhibitor to a suitable carrier that could be targeted to the desired site of action. This has been accomplished with human albumin microspheres (HAM). HAM are nontoxic, nonantigenic and biodegradable, and because of their unique size are trapped in the pulmonary capillary bed after intravenous injection. An effective HL elastase inhibitor has been linked to HAM:

HAM-CONH-Spacer-Ala-Ala-Pro-ValCH_2Cl

The HAM-linked inhibitor was found to be capable of inhibiting elastase. When the inhibitor-HAM were injected into rats, they were rapidly and exclusively taken up by the lungs. Half of the modified HAM remained in the lung with a half-life of ca 17 days (17). Thus HAM offer a method for the delivery of elastase inhibitors to the lung.

Acylating agents. A number of HL elastase inhibitors have been developed that inactivate the enzyme by acylation of the active site serine residue. These include aza-peptides (18) such as Ac-Ala-Ala-Anle-OCH_2CF_3 (Anle = azanorleucine) that react with HL elastase to form stable acyl enzyme derivatives (figure 4). The acyl enzyme (a carbazate) formed from an azapeptide is much more stable to hydrolysis than the acyl enzyme (an ester) formed from a normal peptide substrate due to the electronic influence of the α-nitrogen atom in the azapeptide. In addition to acting as inhibitors, azapeptides such as the 4-nitro-

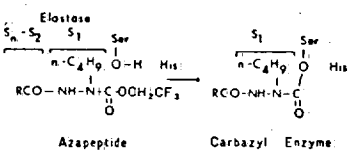


Fig. 4. Reaction of Ac-Ala-Ala-Anle-OCH_2CF_3 with HL elastase. The primary binding site (S_1) of the enzyme is shown interacting with the side chain of the azapeptide. The active site serine of elastase is acylated forming a carbazyl enzyme.

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phenyl ester Ac-Ala-Ala-Anle-$OC_6H_4NO_2$ can be used to determine the active site concentration of HL elastase (19). A group at Merck has also reported a series of azapeptides that do not acylate elastase but act instead as competitive inhibitors (20). For example, Ac-Ala-Ala-Pro-$NHN(CH_2)CO$-$OCH(CH_3)CO$-$NHCH_2C_6H_5$ has a K_I (dissociation constant of the enzyme inhibitor complex) of 2.2×10^{-5} M.

A group of heterocyclic acylating agents for HL elastase have been reported by Merck. These include N-acylsaccharins such as furyl saccharin ($ID_{50} = 3.6 \times 10^{-7}$M), N-acylbenzoisothiazolinones, and N-arylbenzoisothiazolinone, 1,1 dioxides (21, 22). These inhibitors acylate HL elastase by reaction between the active site serine residue of the enzyme and the heterocyclic ring carbonyl group. Some of the inhibitors show good selectivity toward HL elastase and others are fairly broad serine protease inhibitors.

A selective acylating agent for HL elastase could potentially be used therapeutically. A balance between the inherent reactivity of the acylating agent and potential side reactions such as hydrolysis or reaction with nucleophiles and its reactivity with HL elastase must be achieved. Some reagents such as alkyl isocyanates (23) are too reactive and nonselective for therapeutic use. Some azapeptides and acylsaccharins also appear to be unsuitable due to their hydrolytic instability. Thus the ideal inhibitor for further testing does not yet appear to be available.

Sulfonyl fluorides. Sulfonyl fluorides inhibit serine proteases by reacting with the active site serine residue to form a sulfonyl derivative (figure 5). We have discovered recently that introduction of fluoroacyl groups into a sulfonyl fluoride structure gives considerable reactivity and selectivity for HL elastase (24). HL elastase was inhibited most rapidly by 2-$(CF_3CF_2CONH)C_6H_4SO_2F$ ($k_{obs}/[I] = 1700$ $M^{-1}s^{-1}$) that is slightly better than the best peptide chloromethyl ketone for this enzyme. It also was quite selective and reacted with HL cathepsin G and bovine chymotrypsin at rates that were slower by 130- and 57-fold, respectively. One potential disadvantage of these 2-substituted sulfonyl fluorides is their instability toward hydrolysis. Whether this problem can be overcome and a useful agent produced still remains to be seen.

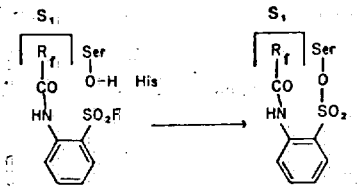

Fig. 5. Reaction of a 2-perfluoroacylamino benzene sulfonyl fluoride with the active site serine residue of HL elastase. R_f can be CF_3, CF_2CF_3, or $CF_2CF_2CF_3$.

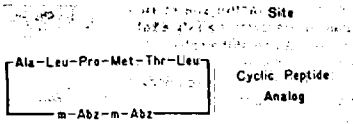

Fig. 6. Comparison of the reactive site sequence of α_1-PI with that of a cyclic peptide analogue. The bridging group is composed of 2 *m*-Abz (*m*-aminobenzoyl) moieties and is quite rigid.

α_1-protease inhibitor analogs. Crystallographic data have not yet been obtained on the plasma protease inhibitor α_1-PI, but X-ray structures have been obtained on several protein protease inhibitors from plant, microbial, and mammalian sources, and on 2 trypsin-trypsin inhibitor complexes. Only a small portion of the 2 inhibitors interact with the enzyme; most of the major contacts occur with the 6 or 7 amino acid residues in the immediate vicinity of the inhibitor reactive site. Comparison of the various protease inhibitor structures revealed a surprising degree of similarity in the conformation of the peptide chain at the various reactive sites even though the inhibitors inactivated proteases with quite different specificities. Therefore it is reasonable to assume that the conformation at the reactive site of α_1-PI bears some similarity to that of the other protease inhibitors. We have designed a number of inhibitors based on this premise.

A cyclic peptide analog of the α_1-PI reactive site is shown in figure 6 (18). This inhibitor was constructed by taking 6 amino acid residues in the α_1-PI reactive site and joining them with a bridging group that would allow those amino acid residues to occupy a conformation similar to that observed by X-ray crystallography in the reactive site of the soybean trypsin inhibitor. We substituted Leu for Ile for synthetic reasons because we decided it would not be a significant change. The Thr substitution for Ser was made at a time when α_1-PI was believed to contain either a Thr or Ser at that position.

The cyclic peptide inhibited HL elastase reversibly ($K_{II} = 0.39$ mM) and was quite specific because it did not inhibit porcine pancreatic elastase. A linear peptide Ac-Ala-Ile-Pro-Met-Ser-Ile-Pro-Pro-NH_2 with the α_1-PI reactive site sequence is a good substrate for HL elastase and has a K_M of 1.6 mM (6). The cyclic peptide binds more tightly to the enzyme than the linear peptide and was not cleaved by HL elastase even after long incubation times. The stability of the cyclic peptide analogs of α_1-PI toward HL elastase makes this class of compounds a potential useful group of inhibitors if sequence substitutions can be made that would lower the K_I value several fold.

Heterocyclic HL elastase inhibitors. HL elastase is inhibited competitively by heterocyclic compounds such as 2-substituted 4H-3,1-benzoxazin-4-ones, 4-quinazolines (figure 7), and 4-chloroquinazolines, N-substituted phthalimides and by thioesters of N-acylanthranilic acids (25). The most potent inhibitors have K_I values in the 10^{-7}-10^{-8} range. The best inhibitors had fluoroalkyl or fluoroacyl substituents. The quinazolinones, chloroquinazolines and N-substituted phthalimides were quite specific for HL elastase. An excellent correlation was observed between pK_I and the infrared carbonyl stretching frequency of the inhibitor. Thus the partially polarized carbonyl group of the inhibitor is interacting with the partially polarized catalytic triad (Ser-His-Asp) of HL elastase. The substituents on the inhibitor probably interact with the primary substrate binding site of elastase. This class of compounds eventually may be useful therapeutically.

Other reversible inhibitors. Quite a variety of other structures have been shown to be HL elastase inhibitors. These include the fermentation products elastatinal [$K_I = 5 \times 10^{-5}$ (26), $K_I = 8 \times 10^{-5}$M (12)] and elasnin [$ID_{50} = 3.3 \times 10^{-6}$M (27)]. Other examples include dipeptides such as 3-CF_3-C_6H_4-CO-Ala-Ala-NH-C_6H_4-NO_2 [$K_I = 4 \times 10^{-6}$M (28)], fatty acids such as oleic acid [$K_I = 9 \times 10^{-6}$M (29)], and sulfated polysaccharides such as Arteparon and pentosan polysulfate SP-54 [$K_I = 10^{-7}$-10^{-8}, uncompetitive inhibition with multiple binding (30, 31)]. None of these compounds appears to offer significant advantages as do HL elastase inhibitors.

Animal Studies

Several different types of synthetic elastase inhibitors have been used in animal studies of emphysema. These have included peptide chloromethyl ketones, furyl saccharine, and trifluoroacetyldipeptides. However, peptide chloromethyl ketones have been the most widely studied.

The chloromethyl ketone Ac-Ala-Ala-Pro-AlaCH_2Cl has been extensively studied in hamsters. Experimental emphysema was induced by intratracheal instillation of porcine pancreatic elastase. The inhibitor was administered intraperitoneally in divided doses before and immediately after instillation of the elastase (32). The inhibitor was shown to have significant antielastase activity *in vivo* and markedly decreased the extent of elastase-induced emphysema. In addition, it was shown that the development of emphysema was dependent on the dose of Ac-Ala-Ala-Pro-AlaCH_2Cl with emphysema being eliminated with 4.1 and 8.0 mg doses and markedly diminished with 1.1 mg (33).

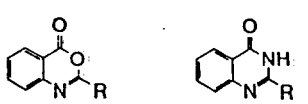

Fig. 7. Structure of a 2-substituted 4H-3,1-benzoxazin-4-one and a 2-substituted 4-quinazoline-one.

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The chloromethyl ketone Ac-Ala-Ala-Pro-AlaCH$_2$Cl was not effective when administered 60 min after exposure to elastase (34). A divided dose of 19 mg was injected intraperitoneally. In addition, this large dose was shown to produce a unique renal tubular nephropathy. In the previous studies when the inhibitor was administered concurrently with the elastase, no evidence of toxicity or kidney injury was observed with a similar dose. The mechanism of this *in vivo* toxicity is not yet known.

The chloromethyl ketone MeO-Suc-Ala-Ala-Pro-ValCH$_2$Cl has been shown to be orally active in protecting Swiss-Webster mice from elastase-induced emphysema (35). The mice were given a transoral dose of porcine pancreatic elastase and were given 300 to 400 μg of the chloromethyl ketone at times ranging from 2 h before instillation of the enzyme to 48 h after the enzyme. Mice that received the elastase inhibitor within 15 min before instillation of elastase were protected from development of emphysema and their lungs were completely free of alveolar deformation. However, no significant protection was afforded by treatment with MeO-Suc-Ala-Ala-Pro-ValCH$_2$Cl 1 h or more before the challenge with the enzyme or at any time after the challenge.

The chloromethyl ketone Suc-Ala-Ala-Pro-ValCH$_2$Cl has been shown by morphometric and physiologic measurements to moderate elastase-induced emphysema in hamsters (36). When 0.5 mg of the inhibitor in saline was injected intratracheally 1 hr before intratracheal injection of porcine pancreatic elastase, the hamsters did not develop emphysema. When the inhibitor was injected 1 hr after the elastase, the severity of emphysema was reduced by approximately 50% compared with control animals receiving saline 1 hr after elastase. The Suc-Ala-Ala-Pro-ValCH$_2$Cl was ineffective when administered intratracheally 4 h after the elastase.

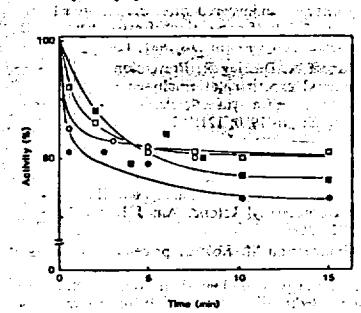

Fig. 8. The effect of elastin on the elastase catalyzed hydrolysis of a synthetic elastase substrate. Elastin (2 mg) was added to a solution of enzyme in 1 ml of saline (0.85% NaCl, 0.02% $CaCl_2$, 0.02% KCl, and 0.002% $NaHCO_3$) at room temperature. At appropriate intervals an aliquot (50 μl) of the suspension was assayed for elastase activity using MeO-Suc-Ala-Ala-Pro-Val-NA (2 mM) as a substrate in 2 ml of 0.1 M Hepes buffer at pH 7.5 containing 0.5 M NaCl and 10% DMSO. HL elastase (2.6×10^{-7} M) was studied with bovine ligamentum nuchae elastin (*open circles*) and dog lung elastin (*closed circles*). PP elastase (1.2×10^{-8} M) was studied with bovine ligamentum nuchae elastin (*open squares*) and dog lung elastin (*closed squares*).

TABLE 1

EFFECT OF ELASTIN ON THE RATE OF INHIBITION OF HL AND PORCINE PANCREATIC ELASTASE BY PEPTIDE CHLOROMETHYL KETONES

Inhibitor	Concentration (μM)	$k_{obs}/[I]$ ($M^{-1}s^{-1}$) No Elastin	Bovine Elastin	Dog Lung Elastin
	Porcine Pancreatic Elastase			
Ac-Ala-Ala-Pro-AlaCH$_2$Cl	4.1	366	181	44
Suc-Ala-Ala-Pro-ValCH$_2$Cl	1.5	685	274	132
MeO-Suc-Ala-Ala-Pro-ValCH$_2$Cl	1.9	630	249	87
	HL Elastase			
Ac-Ala-Ala-Pro-AlaCH$_2$Cl	81	7.4	4.3	7.2
Suc-Ala-Ala-Pro-ValCH$_2$Cl	0.31	1490	198	40
MeO-Suc-Ala-Ala-Pro-ValCH$_2$Cl	0.39	1550	125	71

Elastin (2 mg) was added to 1 ml of elastase in saline (HL elastase, 2.6×10^{-7} M; PP elastase, 1.2×10^{-8} M). After shaking for 10 min, a solution of the chloromethyl ketone in saline was added. At appropriate intervals, aliquots of the suspension were removed and assayed for residual elastase activity using MeO-Suc-Ala-Ala-Pro-Val-NA as a substrate. The k_{obs} was obtained from a plot of ln velocity versus time.

To explore the reasons for the ineffectiveness of peptide chloromethyl ketones when administered after the dose of elastase, we examined the reaction of elastase with substrate and inhibitors in the presence of elastin. When HL elastase or porcine pancreatic elastase is added to excess elastin, the ability of the enzyme to hydrolyze a small synthetic substrate is only slightly diminished (figure 8). This indicates that the elastase molecules bound to elastin are slightly less available to the substrate or that a slight alteration in the active site conformation has occurred. In addition, binding of the elastase to the elastin is complete within a few minutes.

Rates of inhibition of elastase by peptide chloromethyl ketones were then measured in the presence and absence of elastin (table I). In all cases, the chloromethyl ketone elastase inhibitors examined were much less effective at inhibiting elastase preabsorbed onto elastin. Indeed, with the chloromethyl ketones used in the animal emphysema models (porcine pancreatic elastase induced), inhibition rates were decreased by two-to eightfold. Even larger rate reductions were observed with HL elastase preabsorbed onto elastin. These low rates of inhibition may explain the ineffectiveness of peptide chloromethyl ketone elastase inhibitors in the animal models when administered after the dose of elastase. After the elastase has had an opportunity to bind to lung elastin, it is much harder to inhibit. It would be interesting to investigate whether all classes of elastase inhibitor exhibit this type of behavior. In future therapeutic protocols, it may be necessary to maintain a constant presence of elastase inhibitor in order to knock out the enzyme before it can interact with elastin.

Only a few other classes of elastase inhibitors have been studied in animal models. Using the papain-induced emphysema model in hamsters, chaulmoogric acid, furoyl saccharin, the azapeptide Ac-Ala-Ala-Pro-NHN(CH$_3$)CO-OCH(CH$_3$)CONH$_2$, and elastatinal have been shown to be effective inhibitors (37). The dipeptide CF$_3$CO-Lys-Ala-NHC$_6$H$_5$ and the chloromethyl ketone CF$_3$CO-AlaCH$_2$Cl have been tested in the porcine pancreatic elastase-induced emphysema model in hamsters (38). The chloromethyl ketone gave significant partial protection against the emphysematous lesions, but no protection was obtained with the dipeptide. This is an excellent *in vitro* elastase inhibitor and possibly it is cleaved at the Lys residue *in vivo* by some trypsin-like enzyme. Interestingly, it has been reported in an abstract that α_1-protease inhibitor or elastatinal were partially effective at suppressing emphysematous lesions in the hamster-elastase model when the inhibitors were administered 6 days after the elastase instillation (39). This observation should be confirmed independently by other investigators because other types of inhibitors (peptide chloromethyl ketones) have been shown to be ineffective at such a long interval after elastase instillation.

Prospects

It now appears likely that HL elastase is primarily responsible for the destruction of lung tissue observed in pulmonary emphysema. Other enzymes such as cathepsin G may act synergistically with HL elastase or contribute to the damage by other mechanisms. Thus synthetic elastase inhibitors have considerable potential for the treatment of emphysema. Indeed, several classes of compounds have already been shown to be active in animal models of emphysema.

At present, better elastase inhibitors are needed. They should have a high degree of selectivity and minimal side reactions or toxic effects. In addition, more studies are needed on the effect of elastase inhibitors on a variety of animal emphysema models.

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More toxicity data on some of the compounds already tested also are needed. However, the overall prospects are quite good for the development of a synthetic elastase inhibitor for use in humans in the near future.

It is clear that a synthetic elastase inhibitor would be a useful therapeutic agent. Further progress in the field simply awaits the development of a more ideal inhibitor structure.

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